Vaccination pattern affects immunological response

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The response of the immune system to different vaccination patterns is studied with a simple model. It is argued that the history and characteristics of the pattern defines very different secondary immune responses in the case of infection. The memory function of the immune response can be set to work in very different modes depending on the pattern followed during immunizations. It is argued that the history and pattern of immunizations can be a decisive (and experimentally accessible) factor to tailor the effectiveness of a specific vaccine.

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I. INTRODUCTION AND OVERVIEW

From the physical scientist standpoint, the immune system (IS)[1, 2] (with cell-mediated and/or humoral responses) ranks amongst the most complex naturally-occurring nonlinear many-body problems we can find[3]. The IS involves interactions amongst several entities (antibodies (ATB), antigens (ATG), immune complexes (IC), natural-killer (NK) cells, plasma cells (PC), T-cells, B-cells, antigen presenting cells (APC), etc) with complex affinities and dynamics. The understanding of the IS response, even at a qualitative level, is of prime importance for current issues in immunology, ranging from HIV[4] and other immunodeficiencies, to tumor immunotherapy[5]. Some of the components of the IS (like T, NK, or B-cells) have complex internal dynamics of their own, resulting in differentiation, division, and mutation. The dynamics of the IS presents a problem with a hierarchy of complexities at different levels; the IS response is to a cell what sociology is to an individual, having both internal and emergent properties arising from their inherent complexity.

Mathematical modelling in theoretical immunology has come as an aid in the understanding of the IS. This is in part due to the unprecedented growth in computer memory and processor speed, but also due to a better understanding of the dynamics of the IS. Extensive reviews of the early pioneering work in the mathematical modelling of the IS can be found in the literature[3].

Admittedly, in spite of more than two decades of research, the modelling of the IS response is still in its infancy. The shear complexity of the problem is not the only reason, but also the fact that many microscopic interactions among different components are either not fully understood, difficult to measure, or no reasonable parametrization is known for them. There is also the widespread opinion that not all of the fundamental molecules participating in the IS response might have been fully identified[3], in particular compounds related to inter-cell signalling and communication.

Despite all these shortcomings, the development of models is an important advance in our understanding and a clear aid in the development of intuition and strategies to guide the IS in the right direction to combat diseases like AIDS or cancer. Monoclonal antibodies (MA) and interferon based therapies have become nowadays part of the standard repertoire in cancer treatment, while many other types of immunotherapy, such as cancer vaccines (CV), remain largely experimental. CV's consist in most cases of a source of cancer-related material (antigen) which is injected to further stimulate the IS. The experimental challenge so far has been to find better antigens with enough effectiveness to enhance the patient's IS to fight cancer cells.

This paper focuses on a very specific aspect of the IS: the amplitude of the secondary immune response according to different *training* programs established in the vaccination pattern (primary response). In general terms, the most basic task of the immune system is pattern recognition; a task which the IS achieves through the mechanism of *clonal selection*, elucidated more than 40 years ago[6]. Clonal selection prepares the *memory* of the IS to deliver a strong response (the secondary response) if the antigen reappears. The initial steps of the secondary response are crucial to the faith of the organism in its ability to fight a recurring antigen. If the IS has not been trained before, a massive attack from an antigen leaves the organism to rely only on the primary response. This has the disadvantage that there is an associated delay, because the cell population needs to enlarge before substantial amounts of antibodies can be produced. This is partly the reason why illnesses from highly mutating antigens like influenza are more difficult to fight by the IS than more severe, but at the same time more stable, antigens. The former leaves the IS at the mercy of the primary response, while the latter can be more effectively fought

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through vaccination.

The way the IS develops a *memory* is based on its training (vaccination) program. It will be argued that the ability to develop a memory, like in many other pattern recognition problems, is strongly dependent on the type of training. The possibility of playing with the timing and sequence of the vaccination program to tailor and maximize the effectiveness of the IS secondary response is suggested.

II. THE IMMUNE SYSTEM AS A LEARNING MACHINE

On very general theoretical grounds the IS is a learning machine for pattern recognition of the epitopes of antigens. Forrest *et. al*[7] have considered the adaptability of the IS response as a pattern recognition and learning process using a genetic algorithm[8] on a binary string model. After Ref. [8], genetic algorithms are stochastic search methods managing a population of simultaneous search positions; they evaluate the target function to be optimized at some randomly selected points of the definition domain. At this level of abstraction, there is a strong overlap between the learning properties of the IS and many concepts in stochastic neural networks, including learning algorithms and optimization. As far as the experimental evidence is concerned, there is no clear-cut demonstration on the exact algorithm the IS uses to learn. Other options different from genetic algorithms like Boltzmann or Hebbian learning[8] should be considered on an equal foot.

Several radically different types of models have been proposed for the IS, going from coupled systems of (non-linear) differential equations[3, 9, 10], to spin-glasses[11], to cellular automata (CA)[12, 13]. Segel pointed out[14] that a hallmark of complex phenomena is that they can not be modelled by a single approach or, alternatively, that they are prone to several different representations, depending on the specific aspect we want to understand. The aspects of training, learning, and pattern recognition we want to study are more easily implemented on CA versions of the IS. We shall use, accordingly, one of the well established CA models of the immune response[12, 13] to address the issues we raised before, trying to draw analogies with aspects of learning in neural networks where appropriate.

For the sake of argument, consider the case of Boltzmann learning in a simulated annealing process of the type used for neural networks[8]. Simulated annealing, which is a special case of the Monte Carlo method, was brought into the mainstream of numerical optimization of networks after the seminal paper by Kirkpatrick and coworkers[15]. The learning process has here a direct physical meaning: it is equivalent to the previous thermodynamic history of the network in its search for the global minimum of the total energy. This is the clearest example in which it is obvious that the outcome of the learning process strongly depends on the thermodynamic cycles and patterns followed in the annealing. One quick temperature jump followed by a rapid quench will have a completely different effect than a sustained and gradual temperature drop, even if the amount of energy put in is the same. The differences will be more pronounced the more complex the energy landscape or the network. It is objective of this paper to show that the same phenomenon exist in simple models of the IS, thus showing the importance of the pattern followed in the training period (vaccination).

III. THE MODEL

We do not to validate a new model for the IS here. Instead we adopt a well established CA-model for the IS (IMMSIM), first introduced by Celada and Seiden[12] and further developed by Kleinstein and Seiden[16]. We comment very briefly on the model and refer to the published literature[12, 13, 16] for the details. Essentially, the model follows the evolution in a CA (spatial lymph-node) of: antigen presenting cells, antibodies, antigens, B-cells, immune complexes (IC), plasma cells, and T-cells. We use the 8-bit string implementation with the parametrization suggested by Kleinstein and Seiden[16]. It is well known that even the simplest automata with the simplest interaction rules can lead to extremely complex behavior including chaotic dynamics, unstable periods, and complex spatiotemporal patterns[17]. An automata like the one used in IMMSIM has the additional complication that some of its entities change over time in a stochastic manner, from hyper-mutations to the finite lifetime of the cells; its dynamics can only be assessed by direct simulation on a grid. Further details of the model can be found in the original papers[12, 13, 16]. It is assumed that all memory cells (APC, B-, and T-cells) have an infinite lifetime while the non-memory version of the same cells have an average half-life of τ =10 cycles, defined as the probability $P = \exp(-\ln 2/\tau)$ to die in each cycle. The permanence/extintion of a cell is decided stochastically in each iteration, a feature that transforms the IMMSIM model into an stochastic cellular automata. Direct interaction between IC's and B-cells are not considered in the model[12, 13, 16]. The relative shorter lifetimes of B-cells as compared to the total vaccination period makes the total number of B-cells to be dominated mostly by memory B-cells. We shall use, accordingly, the total number of B-cells as a measure of the gained immune memory in the next section.

IV. RESULTS AND DISCUSSION

We adopt a few definitions: We define two periods (a) a vaccination/inoculation or training period in which the CA is trained by exposing it to a small quantity of antigen with different temporal patterns, and (b) an infection

period in which the CA is exposed to much larger quantities of antigen which is injected at regular short intervals in the dynamics; thus simulating a quasi-continuous production of antigen caused by illness or external agents.

We will judge the ability of the IS to overcome infection by its ability to reduce the amount of existing antigen to exactly zero, during the infection period. This is a somewhat arbitrary definition but it will reveal, precisely, the different types of behavior that can be generated in the CA according to the training pattern.

Form the experimental point of view, once an antigen or a modified form of antigen has been produced for a trial, there are very few variables left except for: (a) the amount of antigen to be introduced as vaccine, and (b) the number of inoculations and the length of the training program. The results in this paper suggest that for exactly the same amount of antigen used as a vaccine, the IS may respond in completely different ways depending on how that vaccine is spread over the training period.

We first concentrate on the concepts of infection and primary response for the purpose of the modelling here. Figure 1 shows the example of of a CA which *has not* been vaccinated, i.e. it has no previous recollection of an encounter with the antigen, and it is exposed at some point to an infection in the terms defined above. The CA has a standard population of T-, APC, and B-cells of$\sim 10^3$ and no antigen is present initially. Here we are testing the ability of the model-IS to cope with an infection by means of its primary response only. In a CA the cycles play the role of time; they can be used as synonyms in this context. In Fig. 1(a) the CA is injected from $t = 0$ onwards with 10^4 antigens every 10 cycles. The positions where the antigens are introduced are marked with vertical arrows. In Fig. 1(a) we see that after 4 exposures to the periodic outbreak of antigen the CA has managed to reduce the antigen content to zero. This is a case where, with some delay, the IS is able to control the infection by means of its primary response. On the contrary Fig. 1(b) shows an example where the primary response cannot cope with the outbreak. Here we inject 5×10^4 antigens every 10 cycles and monitor again the amount of antigen present in the CA. After a rapid increase, there is a hint of a slowdown in the amount of antigen present, i.e. the primary response is responding and fighting back partially the infection. But eventually the constant increase of antigen at regular times wins and the amount of antigen increases continuously and cannot be controlled. We are interested now on how a vaccination program with a small amount of antigen can help the IS to cope with this latter situation.

We now define a vaccination program. From the response in Fig. 1 we can deduce that 10^3 iterations is a very long time compared to the response of the CA. In addition, an injection of a total of 10^4 antigens spread over 10^3 cycles can be easily handled by the model-IS. We define a vaccination program, accordingly, based on a total antigen intake of 10^4 (the vaccine) spread in different ways over a period of 10^3 cycles (the training period). Figure 2 shows the result of different vaccination patterns. A measure of the ability of the system to memorize the presence of the antigen for future infections is the total number of B-cells present in the system. As one of the primary targeting centers of antigens, B-cells play a decisive role on the memory of the IS to trigger a fast secondary response. The total number of B-cells is dominated by the memory B-cells, as explained before.

Figure 2 shows then the B-cell population resulting from 3 different vaccination patterns with the same total amount of antigen: (a) a single inoculation with 10^4 antigens at $t = 0$, (b) two inoculations with 5×10^3 antigens at $t = 0$ and $t = 500$ cycles, and (c) 10 inoculations with 10^3 antigens every 100 cycles. It is evident from the B-cell population that the build up of immune memory is very different depending on the pattern followed during vaccination, even if the total amount of antigen is the same in all cases. Smaller doses spread-out over the training period result in better memory retention as far as the B-cell population is concerned; but the picture of the better memory gain with small doses is in fact subtler. A naive conclusion from Fig. 2 would be that we have to continue making the doses smaller and more frequent. But there is in fact a tradeoff between the amount, frequency, and lifetime of the antigens. The gain in B-cell population for 20 inoculations with half of the dose is negligible, for example, and for 50 inoculations the final population of B-cells after vaccination is smaller than in the 10 inoculation program, as we shall show later.

The three different scenarios in immune memory gain in Fig. 2 result naturally in three completely different secondary immune responses in the event of infection. Figure 3 exemplifies this with an infection of the type shown if Fig. 1(a), i.e. 10^4 antigens every 10 cycles. The IS controls the infection now much faster than the~ 35 cycles it takes to the primary response, as expected, but significant differences are seen in the dynamics of the three cases. The single inoculation training controls the infection only after the second antigen injection, while the 2 and 10 inoculations approaches, control the outbreak right after the first appearance of the antigen and before the second injection. During that time the dynamics manages to clear the CA from antigen; the 10 vaccination approach being the fastest. It may seem *a priori* obvious that the more vaccines the better the protection, but let us stress that all vaccination programs are carried out with exactly the same amount of antigen; i.e. more inoculations does not mean more vaccine, but rather the same amount of vaccine distributed differently.

The effect of the different vaccination programs and the different immune memory gains is more dramatic in outbreaks that cannot be controlled by the primary response of the IS. This is explicitly shown in Fig. 4: a CA trained with the vaccination programs of Fig. 2 is exposed now to an infection outbreak of 5×10^4 antigens every 10 cycles. Figures 4(a) and (b) show the responses of the single and double vaccination programs. After an

initial increase, there a sub-linear slowdown in the population of antigen which is a mixture of primary and secondary response. But the outbreak eventually dominates and the antigen population increases out of control from there on. The 10 inoculations program, on the other hand, controls the outbreak immediately after the second antigen injection. We show a few more cycles after the second to demonstrate that the outbreak effectively remains under control and that the model-IS manages to reduce the antigen population to zero in between injections. The difference in the training program during vaccination represents, in this model, the difference between life and death for the IS.

The important question is then how to maximize the memory of the IS, given a certain amount of vaccine. As pointed out before, it is not as simple as spreading out the dose as much a possible over the training period by means of more frequents inoculations with less amount of antigen. Let us first show explicitly the behavior of the CA for more frequent vaccinations. Figure 5 shows the increase in B-cell population for a 20 and 50 inoculations program with the same total amount of vaccine used in Fig. 2. It can be appreciated in the figure that a 20 inoculations program finishes with the same number of B-cells as the training with 10 vaccines (compare with Fig.2) and, moreover, a 50 inoculations treatment results in a smaller number of B-cells than the previous ones. An obvious alternative is a training program with a graded vaccination dose of antigen. Figure 5 shows an example where the 10^4 antigens used as vaccine are spread in four doses of 500, 1500, 2500, and 5500 at $t = 0$, 250, 500, and 750 cycles, respectively. This an other similar vaccination patterns cannot surpass the total immune memory gain of the 10 vaccine training.

From here we are left with the question of: why does the immune memory increases by spreading the vaccination antigen evenly during the training period, up to a maximum number of doses above which the effect reverses? The answer to this comes from a combination of effects: the lifetime of the cells, the primary response, and the cumulative immune memory gained during the vaccination period. The ideal vaccination program should aim at having: (i) a minimum of primary response per vaccination, (ii) a minimum effect of secondary response from the previous vaccines, and (iii)) a maximum memory imprint on future immunological responses. The gain of memory, in particular, becomes more and more difficult when a previous history of vaccination is already present, for the new dose is directly exposed to a secondary response with production of antibodies and rapid elimination of the new antigen. This explains the slowdown in the immune memory gain seen in Fig. 5. For real immune systems, the tailoring of the dose has to be decided experimentally. In particular, it depends strongly on the type of antigen, which is some cases is difficult to obtain (like in cancer vaccines) and may not provoke a full immunological response. A fine balance between the effectiveness of different vaccination programs and the type of antigen can only be achieved through experimental studies on large populations. Real systems have also outbreaks with *continuous* production of antigen rather than the "model-outbreak" studied here. We observed qualitatively similar results by spreading the antigen in the outbreaks in different manners, but it is not obvious in general that a more complex or realistic automata will not display different dynamics depending on the nature of the outbreak.

The important qualitative lesson learnt from the simulations here, however, is that the timing of the vaccination program may provide an additional variable to increase the effectiveness of vaccines. The effect exist, at least, in a basic model of the IS with the essential variables and components.

V. CONCLUSIONS

The effect of different programs of exposure to antigen in a model IS have been investigated with the aim to understand the mechanisms to achieve maximum immune memory for a fixed amount of antigen. The gain of immune memory is a delicate compromise between the increase in the population of defending cells and the generation of antibodies through the fast secondary response which tends to block the effect of further doses. As far as the prediction of simple cellular automata is concerned, the results in this paper demonstrate that multiple small doses of vaccine with the same total amount of antigen can boost the immune memory to the extent that it can make the difference between survival or death for the organism. A separate study of long-term effects of the vaccination program on the immune response is in progress and will be published elswhere[18].

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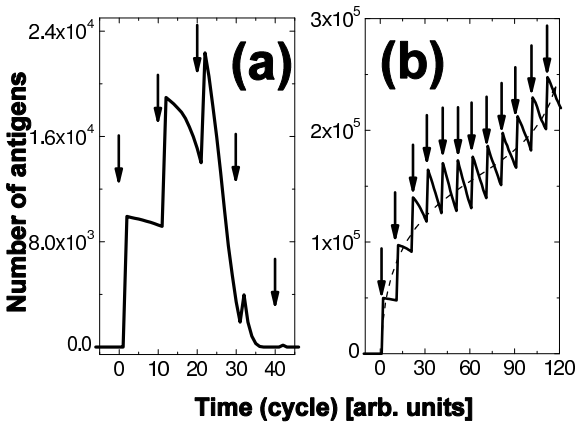

Figure 1: (a) Primary response of an outbreak of 10^4 antigens every 10 cycles in a non-vaccinated CA. The system copes with the outbreak and reduces the amount of circulating antigen to zero after$\sim$ 35 cycles. In (b) the outbreak is of 5×10^4 antigens every 10 cycles. There is a small slowdown in the increase of the antigen population after the initial response of the IS, but the infection eventually wins and the antigen population increases indefinitely. This would be a case of an infection overcoming the capacity of the IS to cope with an unknown antigen only by means of its primary response.

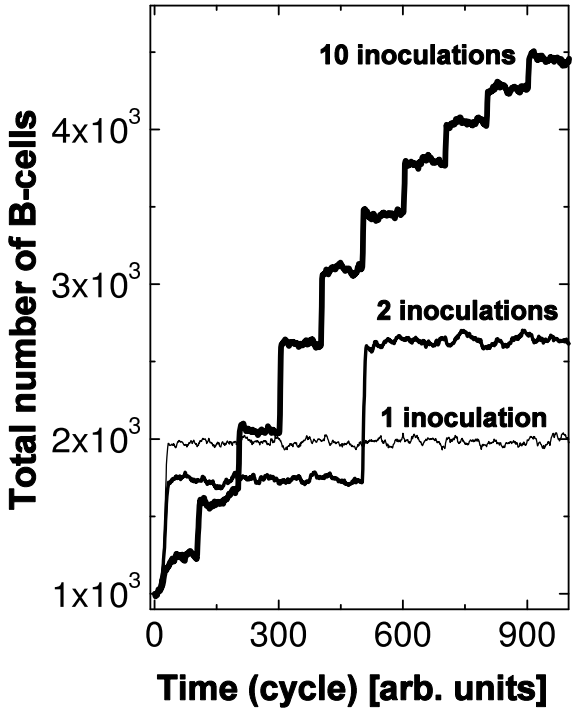

Figure 2: Total number of B-cells as a function of time (cycle) for a 10^3-cycles vaccination program with 10^4 antigens. Three cases are shown: 1 inoculation with the full dose of 10^4 at $t = 0$, 2 inoculations at $t = 0$ and 500 with 5×10^3 antigens, and 10 inoculations with 10^3 antigens every 100 cycles. The total population of B-cells is dominated by memory B-cells which have an infinite lifetime (unlike normal cells with a half-life of 10 cycles). The distributed vaccination program achieves a total memory for a secondary response which is more than twice the memory gained with a single inoculation.

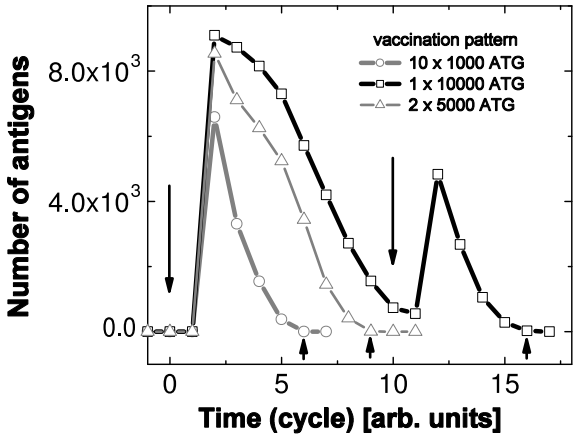

Figure 3: Response of the IS to the infection in Fig. 1(a) after vaccination. For the single inoculation case the infection is controlled only after the second antigen injection around$\sim$ 16 cycles. The small vertical arrows at the bottom of the curves show the places where the infection is controlled for the first time in the three cases. The two and 10 inoculations program control the infection within the first injection, but with marked differences in response time. See the text for further details.

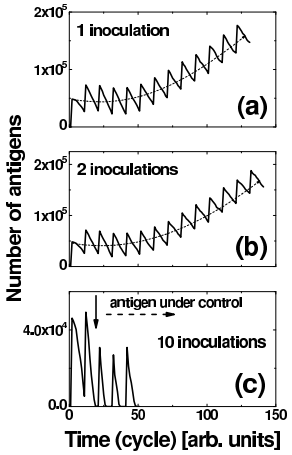

Figure 4: Same as Fig. 3 but for the infection in Fig. 1(b). The three cases of vaccination are shown in different plots for clarity. This is an infection which cannot be controlled by the primary response, as shown in Fig. 1(b). The one and two inoculations programs ((a) and (b)) cannot control the outbreak after vaccination despite a small slowdown in the antigen population at shorter times. The infection prevails in the long run and the antigen quantity increases indefinitely. The 10-dose program (see Fig. 2) in (c) succeeds to control the antigen population after two injections. Further injections of antigen are controlled before the next one comes in; three successive injections after the second are shown in (c). The vertical arrow shows the place where the infection is controlled for the first time. The IS is in complete control of the outbreak in this case. For the *same* amount of vaccine, the difference between life and death for this model IS resides in the way the vaccine is implemented.

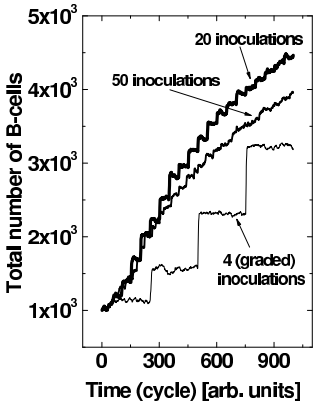

Figure 5: Effect of further dilution in the vaccination doses. A 20 vaccine program attains approximately the same number of B-cells than a program based on 10 vaccines (compare with Fig. 2). A 50-vaccinations programm with the same total number of antigens achieves a total memory on B-cells which is even smaller. The figure shows also a program with four doses of 500, 1500, 2500, and 5500 antigens at $t = 0$, 250, 500, and 750 cycles, respectively. The unsurpassed result of the 10 vaccinations program in Fig. 2 is achieved by a compromise among: small primary response per dose, lifetime of non-memory B- and T-cells and APC's and minimum secondary response from the accumulated memory. See the text for further details.